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Immunologic Studies of Poisonous Anacardiaceae: Oral Desensitization to Poison Ivy and Oak Urushiols in Guinea Pigs

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Poison ivy and oak urushiols or their components were compared with the respective esterified derivatives for efficacy in oral desensitization of Hartley guinea pigs sensitized to urushiols. The esterified derivatives produced a significantly greater degree of hyposensitization than did free urushiol counterparts. Suppression produced by esterified urushiols was of longer duration than that produced by free urushiols. Groups of sensitized guinea pigs were given high (100 mg/kg) or low (10 mg/kg) doses of a mixture of acetylated, saturated urushiol congeners over a 1-, 2-, or 3-week period. High doses produced a greater degree of hyposensitization regardless of the dosage schedule used. Low doses did not produce significant hyposensitization unless given over a shorter (1 week) schedule. Large single booster doses (33 mg/kg/week) of the acetate derivatives produced a rebound in responsiveness when given, 2 weeks following the last dose of the initial series, to animals hyposensitized with 10 mg/kg. No such rebound in sensitivity occurred in animals given a series of high initial doses. The dosage and schedule were also shown to be important considerations for achieving maximal hyposensitization. The most important finding of this study was that sensitized guinea pigs can be desensitized or hyposensitized to poison ivy and oak urushiols via the oral route.

Antigen therapy has been a popular method used by clinicians during this century for attempted reduction of the sensitivity of humans to poison ivy, oak, and sumac (*Rhus*) allergens. The benefits and safety of the use of *Rhus* extracts (containing the active allergenic ingredients, urushiols) for this purpose have been topics of dispute since they were first administered by physicians in 1917. Several reviews pertaining to the clinical use of *Rhus* extracts and allergens have been written [1–3]. From these reviews two salient conclusions emerge. Firstly, *Rhus* extracts should not be used therapeutically, but rather prophylactically. Very small amounts of allergen should be given initially and extreme caution should be used in selecting and escalating the dosage, since urushiols are highly toxic and can exacerbate active dermatitis [3]. Secondly, a measure of hyposensitization in some cases may be achieved but only after prolonged administration and after cumulative doses of 2–4 g of antigen [1]. In hypersensitive subjects, many months, and in some cases over a year, may be required to achieve measurable hyposensitization. Adverse reactions to even minute doses of allergen prevent the rapid escalation of doses of *Rhus* extracts.

The guinea pig is the accepted animal model for studying *Rhus* type allergens since it can be made highly sensitive to urushiols and the cutaneous reactions are similar to those seen in sensitive humans [4]. Susceptibility to sensitization can be blocked in guinea pigs by injections of urushiol components

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Abbreviations:
HDC: 3-n-heptadecylcatechol
MWF: Monday, Wednesday, and Friday
PDC: 3-n-pentadecylcatechol
PIU: poison ivy urushiol
POU: poison oak urushiol

[5], urushiol modified blood cells [6], or urushiol derivatives [7–9] before a sensitizing dose is given. Until recently, no successful desensitization of sensitive guinea pigs has been reported. We have shown that sensitive guinea pigs can be substantially hyposensitized and in some cases desensitized by several intravenous injections of urushiol diacetate esters [7]. Moreover, immune tolerance to sensitization to poison ivy allergen was produced in guinea pigs by a single intravenous injection of urushiol acetate ester before attempted topical sensitization.

Since the intravenous route is objectionable in attempted human desensitization, the present study was undertaken to determine whether orally administered urushiols and their derivatives would hyposensitize guinea pigs to poison ivy and oak urushiols. A comparison of the effectiveness of natural and ester derivatives of urushiols of poison ivy and oak was made to aid in the identification of a product to be used for human clinical trials. A second study was conducted to determine the optimal dosage and schedule for oral desensitization to poison oak urushiol in guinea pigs.

MATERIALS AND METHODS

Animals

Male and female Hartley guinea pigs weighing 300–350 g were obtained from Camm Institute, Wayne, New Jersey, Hilltop Laboratory Animals, Inc., Scottsdale, Pennsylvania, and Glen Tremp, Mundesto, California. Purina guinea pig chow and water supplemented with vitamin C were available ad libitum. The guinea pigs were individually identified by ear tattoo and cage cards.

Urushiol Containing Extracts of Poison Ivy and Oak Plants

Ethanol extracts of *Toxicodendron radicans* (poison ivy) and *T. diversilobum* (poison oak) were obtained from Hollister-Stier Laboratories, Spokane, Washington. The urushiol composition was determined, as previously described [10], to contain 12% and 11.5% urushiol in the ivy and oak extracts, respectively. The composition of poison ivy urushiol (PIU) was 13.6% saturated, 22.4% mono, 63.9% di-, and a trace of triolefin. The poison oak urushiol (POU) composition was 6.8% mono, 10.2% di-, and 83% triolefin.

Synthesis of 3-n-Pentadecylcatechol (PDC) and 3-n-Heptadecylcatechol (HDC)

The PDC and HDC used in some studies were prepared synthetically according to the procedure described for the synthesis of 3-n-nonadecylcatechol [7] using 1-tetradecanal and 1-hexadecanal, respectively, as starting materials for the side chain and 2-3-dimethoxybenzylbromide as the starting material for the catechol.

Poison Ivy and Oak Urushiol Acetate, PDC Acetate, and HDC Acetate

The procedure for preparing diacetate derivatives of poison ivy and oak urushiol, PDC and HDC, has been published [7].

Sensitizing, Desensitizing, and Skin Test Solutions

Sensitizing solutions of poison ivy and oak urushiols were prepared in acetone to contain 1 mg urushiol in 0.15 ml. Desensitizing solutions were prepared in corn oil to contain the equivalent of the active urushiol compound as the free catechol as follows: poison ivy or oak urushiol (PIU or POU, respectively), 5 mg/ml; poison ivy or oak urushiol acetate (PIU or POU acetate, respectively), 5 mg/ml; PDC acetate, 5 mg/ml; HDC acetate, 5 mg/ml; PDC and HDC acetate (1:1) mixture, 1.1, 1.7, 3.3, 11, 17, or 33 mg per ml. Skin test solutions were prepared in acetone and contained 3, 2, 1.5, 1, 0.75, 0.5, or 0.25 μg PIU or POU/5 μl. PDC test solutions contained 1.5, 0.75, and 0.375 μg/5 μl acetone.

Sensitization

Guinea pigs were sensitized by topical application of 1 mg of poison ivy or oak urushiol to the skin over the shoulders and dorsal neck. This dose of urushiol produces a very brisk irritation within 24 h which heals within 1–2 weeks. The sensitization sites were not covered. Two weeks were allowed for sensitivity to develop before initial skin testing.

Skin Testing Procedures

The skin test procedure for evaluating poison ivy and oak sensitivity has been described [7]. Briefly, the hair was removed from the abdominal area with Oster small animal clippers using a #40 blade. Skin test sites of approximately 2.5 cm^2 were delineated on the abdominal skin with a nontoxic marker. Skin tests were of the open patch type. Five microliters of each test solution was dropped from a Hamilton syringe onto the skin within the delineated sites.

The test sites were evaluated at 24, 48, and 72 h after application of test solutions utilizing the scoring system of 0 to +4 ranked intensity for erythema and edema as described by Driaze [11].

The studies were performed single-blind and skin test responses were evaluated by one observer (SW). The responses of each guinea pig were scored individually so that the change in reactivity of each animal could be monitored from one test period to the next.

Ten to 20 nonsensitized negative controls were always included in skin testing as a check for nonspecific chemical irritation.

Oral Desensitization

All guinea pigs were skin tested 2 weeks following sensitization to establish baseline sensitivity before attempted desensitization. Oral desensitization was attempted by administering the test material, dissolved in corn oil, by oral gavage beginning the third week after sensitization. Control animals were dosed with corn oil. Doses were given on Monday, Wednesday, and Friday (MWF) for 1, 2, or 3 weeks. In some cases, the dose was doubled each successive week and in others it was held constant. The total dose administered was 10 or 100 mg/kg of poison ivy or oak urushiol as the free catechol or its equivalent weight in the form of PIU or POU acetate. In some studies, PDC acetate, HDC acetate, or a 1:1 mixture of the two were used for attempted desensitization. The doses administered were based on the equivalent weight for underivatized PDC and/or HDC and were equivalent to 10 or 100 mg/kg of the free catechol.

Effects of Varying Doses of PDC-HDC Acetate and Dosage Schedules on Hyposensitization to Poison Oak Urushiol

Four hundred and fifty guinea pigs were purchased in 3 separate shipments (A, B, C), 150 guinea pigs per shipment. From these animals we were able to obtain 180 animals of sufficient sensitivity for these desensitization studies. All animals were given 1-mg sensitizing doses of POU after arrival and skin tested on the back with 1.5 and 0.75 μg POU. Sixty of the most sensitive animals in each shipment were randomly assigned to 3 groups of 20 animals each for a total of 9 groups.

The experimental design is shown in Table I. One group from each shipment (Groups 1, 4, and 7) served as positive control groups and were orally dosed with corn oil (vehicle). Another group from each shipment (Groups 2, 5, and 8) was given an initial series of doses of PDC-HDC acetate mixture (1:1) totaling 10 mg/kg per animal. A third group from each shipment (Groups 3, 6, and 9) received an initial series of doses of PDC-HDC acetate mixture totaling 100 mg/kg per animal.

One week was allowed between the arrival of each shipment so that

TABLE I. *Protocol to study effects of varying dosages of PDC-HDC acetate, and schedules on skin test sensitivity to poison oak urushiol*

180 Guinea pigs sensitive to 1.5 μg POU or less		
Shipment A	Shipment B	Shipment C
60 guinea pigs, 20 per group, dosed orally on MWF[a] for 3 consecutive weeks (9 equally divided doses):	60 guinea pigs, 20 per group, dosed orally on MWF for 2 consecutive weeks (6 equally divided doses):	60 guinea pigs, 20 per group, dosed orally on MWF for 1 week (3 equally divided doses):
Group 1 1 ml/kg corn oil/day (total dose: 9 ml/kg)	Group 4 1 ml/kg corn oil/day (total dose: 6 ml/kg)	Group 7 1 ml/kg corn oil/day (total dose: 3 ml/kg)
Group 2 1.1 mg/kg PDC-HDC acetate mixture/day (total dose: 10 mg/kg)	Group 5[b] 1.7 mg/kg PDC-HDC acetate mixture/day (total dose: 10 mg/kg)	Group 8[b] 3.3 mg/kg PDC-HDC acetate/day (total dose: 10 mg/kg)
Group 3 11 mg/kg PDC-HDC acetate mixture/day (total dose: 100 mg/kg)	Group 6[b] 17 mg/kg PDC-HDC acetate mixture/day (total dose: 100 mg/kg)	Group 9[b] 33 mg/kg PDC-HDC acetate/day (total dose: 100 mg/kg)

[a] Monday, Wednesday, and Friday.

[b] Groups 5, 6, 8, and 9 were given single booster doses of 33 mg/kg of PDC-HDC acetate per week for 2 weeks, beginning 3 weeks after the last dose of the initial series.

the dosing of animals of Shipment A was begun 1 week before Shipment B and 2 weeks before Shipment C. This resulted in the completion of the initial series of doses on each group on the same day. All animals were then skin tested the week following the last dose of the initial series. The dosage schedules were arranged so that the animals in Shipment A received vehicle or drug in 9 equally divided doses. A dose was administered MWF for 3 consecutive weeks. Animals in Shipment B were given 6 equally divided doses on MWF for 2 consecutive weeks. Shipment C animals were dosed on MWF for a single week, their total dose being divided into 3 equal doses. The week following the last dose of the initial series, all animals were given skin tests of POU (2.0, 1.0, 0.75, 0.50, and 0.25 μg POU) on 5 separate abdominal skin sites. Two weeks following this skin test, animals in Groups 5, 6, 8, and 9 were given a single oral booster dose of 33 mg/kg per week for 2 consecutive weeks in an attempt to provide greater hyposensitization. The week following the last booster dose, the skin tests were repeated on all animals.

RESULTS

Oral Desensitization to Poison Ivy and Oak Urushiols

Two groups of 90 guinea pigs were purchased, one group to be sensitized to POU and the other to PIU. The highly sensitive animals in each group were divided into groups and given oral doses of the respective urushiols or urushiol acetates in an attempt to produce desensitiziation.

Both groups were given 1-mg sensitizing doses of POU or PIU and skin tested 2 weeks later with POU or PIU, respectively. Two additional negative control groups of 20 guinea pigs each were also skin tested with PIU and POU as a check against primary irritancy of the test solutions. The skin test solutions were 3.0, 1.5, 0.75, 0.5, and 0.25 μg of the respective urushiol applied in a volume of 5 μl acetone. Since there is a natural decline in sensitivity of guinea pigs after sensitization, only highly sensitive animals should be selected for desensitization studies. Animals failing to respond with +1 erythema to 0.75 μg doses of PIU or POU were eliminated from the study, leaving 52 guinea pigs in the PIU-sensitive group and 25 in the POU group.

Groups of 13 PIU-sensitive guinea pigs were given one of the following treatments orally: corn oil; Hollister-Stier poison ivy extract (PIU); acetylated Hollister-Stier poison ivy extract (PIU acetate); or 3-n-pentadecylcatechol acetate (PDC acetate). Similar groups (POU-, POU acetate-, HDC acetate-, and vehicle-treated) were planned for the POU-sensitized animals. However, only 25 animals were sufficiently sensitive to POU to be used for attempted desensitization and only 2 groups were established, the vehicle- and HDC acetate-treated groups. Therefore, data from a preliminary study using POU and POU acetate treatments will be presented later in this section of the results.

Oral dosing was begun the week following the initial skin test in both POU- and PIU-sensitive groups. Animals were dosed MWF of each week for 3 consecutive weeks. The animals were given corn oil (1 ml/kg) or 2.5 mg per day of PIU, PIU acetate, PDC acetate, or HDC acetate. The doses were increased to 5 mg per day the second week and 10 mg per day the third week. The total dose of urushiol per animal was 52.5 mg urushiol or the equivalent of 52.5 mg urushiol, in the acetate form.

The week following the last oral dose and 4 weeks later (7 and 10 weeks after sensitization, respectively) the skin tests with PIU or POU were repeated. The percent positive test responses in each group to all test doses of PIU or POU (Table II) were calculated for the following test periods: Pretest, initial skin test before attempted desensitization; Posttest I, first skin test after attempted desensitization; Posttest II, second skin test after attempted desensitization. The percentages were calculated as follows:

$$\%\ \text{positive responses} = \frac{\#\ \text{positive responses in group to all test dilutions}}{\#\ \text{observations/animal} \times \text{number of animals per group}} \times 100$$

In order to illustrate the overall change of sensitivity within a group (no change, hyposensitization, desensitization, increased sensitivity) these changes were tabulated for each animal in each group. The change in sensitivity of each animal was obtained by comparing the highest dilution to which the animal was sensitive before attempted desensitization with that dilution after attempted desensitization. Each animal's change was tabulated according to one of the following criteria: no change; less by 1, 2, 3, or 4 test dilutions; desensitized; increased sensitivity. The percentage of each group showing each change is given in Table III.

In addition to the above 2 methods of comparison, the sensitivity of each group was compared by the method of endpoint dilution as used by Baer and Hooton [4]. The geometric mean dose (μg) required to elicit a positive test response was calculated for each group. In order to calculate the geometric mean it was assumed that if any animal in the group responded to the test substance, that all would respond to a dose 5 times as large as the largest dose [4]. Therefore, animals

TABLE II. *Effects of orally administered poison ivy or oak urushiol esters and free urushiol on skin test sensitivity to poison ivy and oak urushiol in sensitized guinea pigs*

Treatment[a] groups	Skin test periods					
	Pretest		Posttest I		Posttest II	
	% Positive responses[b]	Geometric mean[c]	% Positive responses	Geometric mean	% Positive responses	Geometric mean
Corn oil 9 ml/kg	69 (13)[d]	0.4205	54 (13)	0.6028	50 (13)	0.6707
Poison ivy urushiol 100 mg/kg	74 (13)	0.3864	30 (13)[e]	1.5033	47 (10)	0.8261
Poison ivy urushiol acetate 100 mg/kg	73 (13)	0.3745	20 (11)[e]	2.7382	31 (11)[e]	1.3479
PDC acetate	73 (13)	0.3745	47 (13)	0.9035	36 (13)[e]	1.9255
Corn oil	65 (13)	0.5419	47 (12)	0.8174	37 (12)	1.2408
HDC acetate	66 (12)	0.5274	33 (12)[f]	1.9130	24 (10)[f]	1.9873

[a] Treatment refers to cumulated doses of corn oil, ivy or oak urushiol or their equivalent in the acetate form. Doses were given during a 3-week period between Pretest and Posttest I. See text for details.

[b] The test responses to 3.0, 1.5, 0.75, 0.5, and 0.25 μg poison ivy or oak urushiol were recorded 24, 48, and 72 h after application to test sites. The number of positive responses of each group was divided by the number of observations (15 per animal × number of animals per group) to calculate % positive test responses.

[c] Geometric mean dose (μg) required to elicit a positive skin test response. See text for description of geometric mean.

[d] Number in brackets = n.

[e] $p < 0.001$, chi square analysis.

[f] $p < 0.01$, chi square analysis.

TABLE III. *Comparison of changes in sensitivity after attempted desensitization in guinea pigs treated orally with corn oil or poison ivy and oak urushiols or their acetate derivatives*

Treatment group	Percent of group with change in sensitivity[a] as compared to pretest sensitivity						
	No change	Hyposensitization				Desensitized	Increased
		Less by 1 test dilution	Less by 2 test dilutions	Less by 3 test dilutions	Less by 4 test dilutions		
Corn oil							
Posttest I[b]	15.5	38.5	23	0	0	0	23
Posttest II	38.5	23	7.5	7.5	7.5	0	15
PIU acetate							
Posttest I	9	9	36	27	0	18	0
Posttest II	9	0	27	9	0	27	27
PIU							
Posttest I	27	18	18	18	0	9	9
Posttest II	0	30	30	10	0	10	20
PDC acetate							
Posttest I	38	31	7.5	15	0	7.5	0
Posttest II	15.4	7.5	15.4	0	0	38.5	23
Corn oil							
Posttest I	33	41.6	8.3	8.3	0	0	8.3
Posttest II	25	8.3	25	0	8.3	8.3	25
HDC acetate							
Posttest I	18	9	27.3	18	0	0	27.3
Posttest II	10	20	30	0	10	20	10
Corn oil							
Posttest I	75	25	0			0	0
Posttest II	75	0	0			25	0
POU							
Posttest I	60	20	20			0	0
Posttest II	80	0	0			20	0
POU acetate							
Posttest I	16.6	16.6	33.3			33.3	0
Posttest II	0	16.6	16.6			66.6	0

[a] Percent change in sensitivity was determined by tabulation of the number of animals in each group with the indicated change, dividing by the number of animals tested, and multiplying the quotient by 100. Animals not responding to the highest test dose were considered desensitized.
[b] Posttests I and II are the first and second skin tests after attempted desensitization, respectively.

not responding to the highest dose (3 μg) were assigned a response to 5 times that dilution (15 μg). Draize scores were recorded and evaluated but are not shown for purposes of brevity.

A gradual decline in sensitivity of the corn oil control groups over the 10 weeks of study was observed (Tables II, III) as evaluated by decreasing % positive responses and increased geometric means with time. This phenomenon has been reported [6], and is the reason why desensitization studies to poison ivy and oak in guinea pigs require highly sensitive animals at the initiation of the experiment. A substantial decrease in the sensitivity of the PIU- and PIU acetate-treated groups was seen 1 week following the last oral dose (Posttest I, $p < 0.001$ by chi square analysis). The geometric mean of the PIU acetate-treated group was almost twice as large as that of the PIU-treated group and 4.5 times as large as the corn oil control group, indicating a greater efficacy of the PIU acetate in reducing sensitivity. Ninety percent of the PIU-treated animals showed a decrease in sensitivity (Table III), 2 of 11 animals were completely desensitized 1 week following the completion of dosing, and no animals became sensitive to a higher dilution. Although the control group decreased slightly in overall sensitivity as indicated by slightly elevated geometric mean, 23% of the animals became sensitive to a higher dilution, 61% had sensitivities less by 1 or 2 dilutions, and none of the control animals was desensitized.

The PDC acetate-treated group was not substantially changed in sensitivity until Posttest II when both PIU acetate- and PDC acetate-treated groups were significantly less responsive than the control ($p < 0.001$, Table II). Five of 13 animals in the PDC acetate-tested group were desensitized at Posttest II. The PIU-treated animals had regained their sensitivity at Posttest II to the level of the control (Table II).

Animals treated with HDC acetate were 2.3 times less sensitive at Posttest I and 1.6 times less sensitive at Posttest II by comparison of geometric means with those of the control group (Table II). Chi square analysis of the % positive vs negative test responses in the HDC acetate-treated and control group showed significant differences ($p < 0.01$) at both Posttest periods. At Posttest II, 50% of the control group were less sensitive than they were at Pretest while 80% of the HDC acetate-treated animals had decreased endpoint dilutions. Two of the 10 animals treated with HDC acetate were desensitized. HDC acetate did not produce as high a rate of desensitization to POU as did PIU acetate and PDC acetate to PIU.

A preliminary study in a small group of animals (15 guinea pigs) highly sensitive to PIU was conducted and animals were dosed orally with corn oil, POU, or POU acetate according to the same dosage and schedule utilized in the above experiments. The animals were sensitized with 1 mg PIU and skin tested with 1.5, 0.75, and 0.375 μg PDC before and twice after attempted desensitization. The sensitivity of the POU-treated group did not differ from the control group at any test period (Table IV). The POU acetate animals were substantially less sensitive than the controls at both Posttest periods ($p < 0.001$) and this difference was reflected in the frequency of positive test responses to PDC and increased geometric means as compared to controls (Table IV). Two of 6 POU acetate-treated animals were desensitized at Posttest I as compared to no desensitized animals in the POU and control groups (Table III). Three of 6 POU-treated animals were hyposensitized as compared to 1 out of 4 of the controls and 2 out of 5 of the POU-treated animals at Posttest I. At the final skin test 4 out of 6 were desensitized as compared to one each of the controls and POU-treated animals. The remaining 2 animals in the POU acetate group were hyposensitized while none of the control and POU-treated animals was hyposensitive as compared to pretest endpoint dilutions.

Effect of Varying Doses of PDC-HDC Acetate and Dosage Schedules on Extent of Hyposensitization to Poison Oak Urushiol

The percent of positive test responses to all test doses of POU at each skin test period in groups of guinea pigs given cumulative oral doses of 0, 10, or 100 mg/kg of PDC-HDC acetate mixture (1:1) over a 3-, 2-, or 1-week period are shown in Table V. The percent of positive test responses was calculated as described in the previous experiments. An average Draize score to the various test doses and a mean average Draize score to all 5 dilutions was calculated for each group.

A gradual decline in the sensitivity of the vehicle-treated groups was seen over the 3 test periods (Table V). Administration of 10 mg/kg PDC-HDC acetate over a 3-week period did not produce an acceleration in this decline in sensitivity. However, comparison of the number of positive vs. negative test scores in the control vs. 100 mg/kg PDC-HDC acetate-treated group at Posttest I showed highly significant ($p < 0.001$, chi square analysis) decreased sensitivity. There was no further decline in the sensitivity of the 100 mg/kg group at Posttest II. The level of sensitivity in the 10 mg/kg PDC-HDC acetate group did not differ from that observed in the control group at any test period, showing that the low dose distributed over a 3-week period had no suppressive effect. The average Draize scores to the test solutions (Table VI) showed the same relationships as did the frequency data.

The skin test reactivity of the 3 groups on the 2-week dosing schedule is shown in Table V. Unfortunately, a very weak sensitivity in the control group makes interpretation of this data difficult. However, it appears that large booster doses of PDC-HDC acetate (33 mg/kg/week for 2 weeks) to the low-dose group may have caused an increase in the sensitivity of these animals ($p < 0.001$ by chi square analysis of Posttest I vs. Posttest II responses of this group). An increase in the mean Draize score for this group was noted at Posttest II (Table VII).

The effect of administering high and low doses of PDC-HDC acetate over a 1-week period is shown in Table V. When 10 mg/kg PDC-HDC acetate was administered in 1 week, a 57% decrease in frequency of positive responses from the Pretest frequency was observed vs. a 19% decrease in frequency of responses in the control group from their Pretest responses. Chi square analysis of the frequency of positive responses in this group and the control at Posttest II showed significant decreased responsiveness ($p < 0.001$). Administration of high doses over a 1-week period produced the same degree of hyposensitization ($p < 0.001$ when compared to control). However, 2 booster doses of 33 mg/kg/week administered to the 10 mg/kg group produced a rebound in responsiveness which was significant ($p < 0.001$) in comparison to the control group at Posttest II. The Draize scores of this group (Table VIII) were also extremely elevated at Posttest II. The rebound effect was hardly noticeable in the 100 mg/kg group, and chi square analysis showed that the 4% increase in frequency of the positive

TABLE IV. *Effects of oral administration of POU acetate and free POU on skin test sensitivity to PDC in sensitized animals*

Treatment[a] group	Skin test periods					
	Pretest		Posttest I		Posttest II	
	% Positive responses[b]	Geometric mean[c]	% Positive responses	Geometric mean	% Positive responses	Geometric mean
Corn oil 9 ml/kg	92 (4)[d]	0.375	75 (4)	0.4459	67 (4)	0.9429
POU acetate 52.5 mg	83 (6)	0.375	33 (6)[e]	1.556	11 (6)[e]	6.203
POU 52.5 mg	78 (5)	0.375	56 (5)	0.75	56 (5)	0.9007

[a] Treatment refers to cumulated doses of corn oil, poison oak urushiol or its equivalent in the acetate form. Doses were given during the 3-week period between Pretest and Posttest I. See text for details.
[b] Percent positive responses to 1.5, .75, and .375 μg doses of PDC and geometric means were calculated as in Table II, footnotes *b* and *c*.
[c] Geometric mean dose (μg) required to elicit a positive skin test response. See text for description of geometric mean.
[d] Numbers in brackets = n.
[e] $p < 0.001$, chi square analysis.

TABLE V. *Effect of varying dosages and schedules on skin test sensitivity to poison oak urushiol in PDC-HDC acetate treated guinea pigs*

Treatment[a] group	Schedule[b]	Percent positive test responses to poison oak urushiol[c]		
		Pretest	Posttest I	Posttest II
Corn oil 9 ml/kg	MWF × 3 wk	77 (20)[d]	57 (20)	38 (19)
PDC-HDC acetate 10 mg/kg	MWF × 3 wk	77 (19)	59 (19)	41 (19)
PDC-HDC acetate 100 mg/kg	MWF × 3 wk	81 (20)	40 (17)[e]	38 (17)
Corn oil 6 ml/kg	MWF × 2 wk	50 (19)	30 (19)	29 (19)
PDC-HDC acetate 10 mg/kg	MWF × 2 wk	63 (19)	31 (19)	48 (16)
PDC-HDC acetate 100 mg/kg	MWF × 2 wk	73 (20)	50 (18)	48 (17)
Corn oil 3 ml/kg	MWF × 1 wk	63 (20)	51 (20)	46 (19)
PDC-HDC acetate 10 mg/kg	MWF × 1 wk	75 (20)	32 (20)[e]	64 (18)[e]
PDC-HDC acetate 100 mg/kg	MWF × 1 wk	63 (20)	36 (20)[e]	40 (19)

[a] Treatment refers to cumulated doses of PDC-HDC acetate or corn oil. Doses were divided equally to obtain total dose.
[b] Doses were administered on MWF for 1, 2, or 3 consecutive weeks. Test animals on 1- and 2-week schedules were given additional 33 mg/kg doses on 2 consecutive weeks before posttest II.
[c] The test responses to 2, 1, 0.75, 0.50, and 0.25 μg POU were recorded 24, 48, and 72 h after application to test sites. The number of positive responses of each group were divided by the number of observations (15 per animal × number of animal in group) to calculate % positive test responses.
[d] Number in brackets = n.
[e] $p < 0.001$, chi square analysis.

TABLE VI. *Sensitivity of animals before treatment (Pretest) and after dosing for 3 weeks (Posttest I and II) with PDC-HDC acetate or corn oil*

Treatment[a] group	Skin test period	Average Draize scores to skin tests (μg) of poison oak urushiol						
		2.0	1.5	1.0	0.75	0.50	0.25	$\bar{X}$[b]
Corn oil control (I)	Pretest		3.35		2.40			2.88
	Posttest I	3.25		2.33	1.90	1.58	0.93	2.00
	Posttest II	2.23		1.65	1.32	0.88	0.39	1.29
PDC-HDC acetate 10 mg/kg (II)	Pretest		3.07		2.35			2.71
	Posttest I	3.75		2.82	1.96	1.72	0.51	2.15
	Posttest II	2.49		1.74	1.19	0.72	0.18	1.26
PDC-HDC acetate 100 mg/kg (III)	Pretest		3.05		2.38			2.72
	Posttest I	2.67		1.63	1.02	1.14	0.18	1.33[c]
	Posttest II	2.47		1.69	1.37	0.61	0.10	1.25

[a] All groups were administered vehicle or drug 3 times weekly for 3 consecutive weeks.
[b] $\bar{X}$ = mean Draize score to all doses used for testing during given test periods.
[c] $p < 0.001$ by chi square analysis of number of positive vs negative skin test responses in the treated and control groups.

TABLE VII. *Sensitivity of animals before (Pretest) treatment and after dosing for 2 weeks (Posttest I and II) with PDC-HDC acetate*

Treatment[a] group	Skin test period	Average Draize scores to skin tests (μg) of poison oak urushiol						
		2.0	1.5	1.0	0.75	0.50	0.25	$\bar{X}$[b]
Corn oil control (I)	Pretest		1.73		0.73			1.23
	Posttest I	2.04		1.35	0.89	0.47	0.14	0.98
	Posttest II	1.74		1.17	0.63	0.33	0.05	0.90
PDC-HDC acetate 10 mg/kg (II)	Pretest		2.57		1.25			1.91
	Posttest I	2.07		1.39	1.16	0.84	0.36	1.16
	Posttest II	2.25		1.56	1.13	0.83	0.50	1.25
PDC-HDC acetate 100 mg/kg (III)	Pretest		2.85		1.38			2.11
	Posttest I	2.75		1.98	1.56	1.29	0.43	1.60
	Posttest II	2.76		1.78	1.96	0.88	0.47	1.38

[a] All groups were administered vehicle or drug 3 times weekly for 2 consecutive weeks.
[b] $\bar{X}$ = mean Draize score to all doses used for testing during given test period.

TABLE VIII. *Sensitivity of animals before (Pretest) dosing and after dosing for 1 week (Posttest I and II) with PDC-HDC acetate or corn oil*

Treatment[a] group	Skin test period	Average Draize scores to skin tests (μg) of poison oak urushiol						
		2.0	1.5	1.0	0.75	0.50	0.25	$\bar{X}$[b]
Corn oil control (I)	Pretest		2.43		1.31			1.87
	Posttest I	3.38		2.80	1.87	1.42	0.96	2.09
	Posttest II	3.23		2.56	2.00	1.63	1.12	2.11
PDC-HDC acetate 10 mg/kg (II)	Pretest		2.40		1.35			1.88
	Posttest I	2.93		2.33	1.82	1.32	0.86	1.85
	Posttest II	3.86		3.08	2.33	1.84	0.80	2.38[c]
PDC-HDC acetate 100 mg/kg (III)	Pretest		2.00		0.87			1.44
	Posttest I	2.50		1.55	0.77	0.57	0.17	1.11[c]
	Posttest II	2.61		1.45	1.09	0.75	0.33	1.25

[a] All groups were administered vehicle or drug 3 times weekly for 1 week.
[b] $\bar{X}$ = mean Draize score to all doses used for testing during given test period.
[c] $p < 0.001$ chi square analysis.

responses from Posttest I to II was insignificant. However, the mean Draize scores for this group were also slightly elevated.

DISCUSSION

The most important finding of this study is that guinea pigs can be desensitized or substantially hyposensitized to poison ivy and oak dermatitis by oral dosing. Esterified urushiols were found to be more effective desensitizers than free urushiols. The acetates of the saturated urushiol congeners, PDC and HDC, were also effective desensitizers. The olefinic urushiol congeners have been shown to be more potent as elicitors than are the saturated congeners [6] and the antigenic potency is increased as the number of double bonds in the alkyl side chain increases. It has been generally held that antigenic potency and immunosuppressive potency are linked and that the best sensitizers are also the best tolerizing agents [9]. The findings of this study appear to support that relationship. If this is true, then the ideal substance to be used clinically for human desensitization would be the acetylated triolefinic urushiol congeners. However, the only means presently available for obtaining triolefinic congeners for desensitization is by plant extraction and chromatographic separation. This process is not economically feasible due to the difficulty in obtaining plant material and the expense of solvent extraction. Synthesis of the unsaturated congeners, particularly the di- and triolefins, would require elaborate and expensive procedures. Additionally, olefinic materials are considered to be less stable than the saturated congeners. Therefore, the saturated congeners are more likely to be used clinically than are their unsaturated counterparts.

High oral doses (100 mg/kg) of the diacetate esters provided a greater level of desensitization than low doses (10 mg/kg). Low cumulative doses provided significant desensitization when given over a 1-week period but not if the dose was spread equally over a 3-week period. Large doses provided good hy-

posensitization when given over a 1- or 3-week schedule. Larger booster doses after initial desensitization appeared not to affect immunosuppression when given to animals desensitized on a high-dose schedule. However, large booster doses given to animals desensitized using a low-dose schedule produced a prominent rebound in sensitivity. These findings indicate that clinical studies to determine the optimal dose and dosage schedule in humans will be needed.

Relatively large oral doses (100 mg/kg; or approximately 50 mg/animal) were required to produce hyposensitization, while in previous studies [7] intravenous doses of 6 mg PIU:POU acetate mixture (1:1) produced hyposensitization and 16 mg desensitized 54% of treated animals to 3.2-μg doses of PDC. The remaining animals were markedly hyposensitized. These findings are in agreement with clinical findings in humans showing that smaller cumulative doses of oleoresin are required for maximal hyposensitization via the parenteral route as compared to the oral route [1]. The requirement of larger oral as compared to parenteral doses to produce substantial hyposensitization is probably due to poor gastrointestinal absorption of urushiols from corn oil solutions [12]. The pattern of hyposensitization observed in our animal studies resembles that seen in humans; namely, hyposensitization is first noticed by a decrease in sensitivity to the highest dilutions. With time and continued administration of allergen the hyposensitization develops to the stronger concentrations until the animals are maximally hyposensitized or desensitized. The data in Table III illustrate this pattern most effectively.

The waning of hyposensitization with time that often occurs in humans after cessation of dosing has not been observed in our animal studies. However, long-term follow-up studies in guinea pigs are hampered by the gradual natural decrease in sensitivity of the animals beginning 6 to 8 weeks following sensitization. For this same reason the benefits of maintenance doses of urushiol acetates in guinea pigs cannot be easily determined.

In addition to the superiority of esterified urushiol derivatives as desensitizing agents, comparative LD_{50} studies, local tissue reactions, and skin irritation studies (data to be published elsewhere) showed that the esterified urushiols are substantially less toxic than their underivatized counterparts. The fact that the esterified urushiols are more effective desensitizers and less toxic points to a better therapeutic index for the urushiol esters.

There have been clinical reports attempting to associate desensitization therapy with renal toxicity [13]. However, a chronic 6-month study in rats dosed orally with free and esterified urushiols (data to be published elsewhere) showed no renal toxicity with either form. Additionally, we have not observed any toxic effects from administration of the free or esterified urushiol in guinea pigs. Moreover, we have not been able to elicit perianal dermatitis in guinea pigs even when 3-μg test doses of urushiol are applied directly to the anus. However, the guinea pig and rat may not be ideal animals in which to study the systemic toxic effects of urushiols since gastrointestinal irritation and perianal dermatitis have been observed in humans after administration of free urushiols.

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